PRELIMINARY REPORT UPON A CRYSTALLOGRAPHIC STUDY OF THE HEMOGLOBINS: A CONTRIBUTION TO THE SPECIFICITY OF CORRESPONDING VITAL SUBSTANCES IN DIFFERENT VERTEBRATES.

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(*Read April 24, 1908.*)

The primary object of this research was to determine whether or not corresponding proteins are identical in different species. Hemoglobin was selected as a favorable substance to begin such a study upon because of its being readily obtained in a state of comparative purity, and, in many cases, readily isolated in crystals. When a sufficient supply of blood was available, it was nearly always possible, by the use of suitable methods, to produce well formed crystals that could be satisfactorily examined and studied by the method adopted. The crystallographic method was chosen because, by its means, differences in substances may be observed that would elude the ordinary methods of analysis employed by the chemist. Moreover, it is comparatively rapid and therefore well adapted to the study of a substance so liable to alteration as hemoglobin. In the method employed it was not even necessary to remove the crystals from the mother liquor for examination. In studying the crystals and measuring the crystallographic constants the petrographic microscope was used, but in the case of these crystals of hemoglobin we have this advantage over the petrographer in his examination of rock sections, in that these crystals are not imbedded in an opaque or semi-opaque matrix, but are in a transparent medium and are usually isolated from each other. Moreover, hundreds and often thousands of crystals are open to observation in a single slide, and these present almost all possible orientations, allowing the optical characters to be determined with much greater accuracy than

is usually the case with minerals in rock sections. Measurements within the limit of error of the instrument could frequently be obtained, and, as various orientations were available, the results of the angular measurements often furnished complete data for the calculation of the axial ratios. The crystals examined were usually complete and often geometrically perfect, so that the symmetry and crystal habit could be determined as readily as in the case of ordinary mineral substances occurring in isolated crystals.

A chemical substance, possessing a rational composition, tends to arrange its parts in an orderly manner so that a definite structure is assumed, which results in a definite external form. This is so universally true that the crystalline condition is the normal one for matter of definite composition. Differences of crystalline form hence indicate differences of substance; and, by the crystallographic method of investigation, obscure differences, such as those between isomerides, may readily be detected.

Photographic records of the crystals were secured and upwards of 2,500 negatives have been made. The hemoglobins of more than one hundred species have been examined and data secured in regard to their crystals. From a study of these records certain facts stand out very prominently.

1. *The Constancy of Generic Characters in the Crystals.*—The crystals of the species of any genus belong to a crystallographic group. When their characters are tabulated, they at once recall the crystallographic groups of minerals. The crystals of the genus *Felis* form an isomorphous group; as strictly isomorphous, in fact, as the group of the rhombohedral carbonates among minerals. The genus *Canis* is even more strictly isomorphous, but the crystals of hemoglobin from the two genera are perfectly distinct, the one from the other.

As an example of the individuality of these generic characters the following may be cited: A sample of blood, marked as that of a certain species of baboon was received from one of our Zoölogical Gardens. Upon making preparations and examining the crystals, it was at once evident that they did not correspond to any species of

baboon thus far examined, nor did they show the characters of the genus *Papio*. They were identified by their crystallographic characters as belonging to the cats (genus *Felis*) but not to any species that we had examined up to that time. Inquiry at the Zoölogical Garden from which the blood was received showed that the animal recorded as being subjected to a *post-mortem* examination on the date when the blood was collected was a species of the genus *Felis*, but not one of which we had previously examined the blood. Other similar cases of incorrect labelling of specimens were detected, in which the wrongly labelled blood was one that had been examined and the species known from other specimens.

2. *Specificity in the Crystals of a Genus.*—The crystals of the different species of a genus, when they are favorably developed for good measurement, can usually be distinguished from each other by definite differences of angle, etc.; while preserving their isomorphous character as belonging to a definite genus. In cases where, on account of difficulty of measurement, the differences cannot be given a quantitative value, variations in the habit of the crystals and in their mode of growth will often show specific differences.

3. *The Occurrence of Several Types of Crystals of Oxyhemoglobin in Many Species.*—In some species the oxyhemoglobin is *dimorphous* (crystallizing in two systems or with two axial ratios), in other cases even *trimorphous*. Where several types of crystal occur in this way in the species of any genus, the crystals of *each type* may be arranged in an isomorphous series. In other words, certain genera are isodimorphous or isotrimorphous.

4. *The Constant Recurrence of Certain Angles, Plane or Dihedral, in the Oxyhemoglobin, Hemoglobin and the "Methemoglobins" of Various Species, even when these Species are Widely Separated Zoölogically and when their Crystals Belong to Various Crystal Systems.*—This appears to indicate a common substance in hemoglobins or a common structure in the various hemoglobin molecules.

5. *The Constant Recurrence of Certain Types of Twinning in the Hemoglobins, and the Prevalence of Mimosie in these Crystals.*—This also indicates a common structure in the various hemoglobin molecules.

6. *Differences between Oxyhemoglobin and Reduced Hemoglobin in Certain Species.*—Undoubted differences between the crystals of these two substances in the same species have been observed.

We have gathered additional evidence that other corresponding proteins, as well as certain fats and carbohydrates, will be found to exhibit similar specificities.

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April 23, 1908.

PROC. AMER. PHIL. SOC., XLVII. 189 T, PRINTED SEPTEMBER 26, 1908.